HABC Gentoype Data Submission Form

Submitting Investigator	
Name	Wen-Chi Hsueh
Affiliation	UCSF
Phone	415/885-7397
Lab Contact	Dr. Christian Vaisse
Lab Contact Phone	415/514-0530
Date Submitted	5/18/2005
Polymorphism Information	
Name	3' UTR insertion variant
RS# or Unique Identifier	N/A
Gene	Leptin Receptor (*LEPR*)
Chromosome position	chr1:65814779
Alleles	-/cttta
Assay Information	
Genotyping Method	Template-directed dye-terminator incorporation assay with fluorescence polarization detection (FP-TDI). Reference: Hsu TM, et al. *Biotechniques*. 2001 Sep;31(3):560-8
Genotypes in HWE (Y/N) (attach HWE Form)	Yes. Caucasian genotypes were 1106: 513: 82 (del/del: del/ins: ins/del). African American genotypes were 625: 499: 90 (del/del: del/ins: ins/del).
Amount of DNA used	5 ng/assay
PCR primers	GGACAGTTGCTCACACTTTGTAG GACTCTGGTTTCTTTTCTCTCTCC
PCR Components **and** Concentrations (Taq, buffer, $MgCl_2$, primers, DMSO, other reagents)	10 x Platinum Taq buffer (Invitrogen) 0.5 μL 50 mM MgCl2 0.25 μL 10 mM dNTPs 0.25 μL Primers (10 mM) 0.012 μL each Platinum Taq (Invitrogen) 0.02 μL ddH2O 4.956 μL **TOTAL** **6 μL**

PCR Cycling conditions (time and temperature for each step, # of cycles, etc.)	PCR program: SNPPCR45 95 for 2:00min 92 for 10s 58 for 20s 68 for 30s GoTo 2 44 Times 68 for 10:00min 4 Forever end PCR cleanup program (SAP) 37 for 45min 90 for 15min 4 forever end TDI program: for 20 cycles which is standard. For some assays we may use more or less cycles, between 5 and 80, for optimum result TDI_20 95 for 2.00min 95 for 15s 55 for 30s Go to 2 19 times 4 forever end
Detection Oligo(s) (if applicable)	TTGTGTTATAATGGGTAATATAAAGTGTAATAGATTA
Other Reaction Conditions (detection reaction components, incubation conditions, gel %, etc.)	The genotyping method was FP-TDI. After the PCR, the reaction was treated with shrimp alkaline phosphatase (SAP) for 90 minutes at 37°C. Subsequently, an appropriate dye terminator reaction was then initiated, containing 2 μL of 10 x reaction buffer, 1 μL of the dye terminators (in this case, C/T), 0.05 μL of the detection oligo (10 mM), 0.05 μL of Acyclopol, and 9.9 μL of ddH_2O. The samples were subjected to the extension reaction (TDI program as noted above) and genotypes were read in a plate reader.
Other Assay Info	